

SOMATIC HYBRIDIZATION IN *PUCCINIA GRAMINIS* VAR. *TRITICI*.

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*Synopsis.*

Further studies have been made on somatic hybrids in *Puccinia graminis* var. *tritici* resulting from a mixture of spores of two parental strains on screening varieties of wheat. It is concluded that nuclear exchange alone cannot explain the diversity of strains obtained. A nuclear fusion process followed by recombination and segregation to form dicaryons is suggested to explain this happening.

Somatic hybridization is shown to be important in the field since a prevalent field strain was isolated from a mixture of spores from two other field strains. Sexual hybridization on barberry, mutation and somatic hybridization are now to be regarded as three important causes of variability in *P. graminis* var. *tritici*.

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It has now been established that strains of *Puccinia graminis* Pers. var. *tritici* (Eriks. and E. Henn.) may undergo some form of hybridization when grown in association on wheat seedlings under laboratory conditions (Nelson *et al.*, 1955; Nelson, 1956; Watson, 1957). When selected strains are used and an efficient screening procedure is available it can be shown that the process is of relatively frequent occurrence and highly pathogenic strains may appear in the progeny of avirulent parents (Watson, 1957). The mechanism allowing such hybridization to occur is not known. Nelson (1956) concluded that hybrids were arising by an exchange of nuclei in the parents and Watson (1957) suggested, as a result of the diversity of strains recovered from a mixture of two parents, the possibility of a sexual process similar to parasexualism. Recently in studies with the leaf rust organism Vakili and Caldwell (1957) have concluded that nuclear exchange cannot explain the multiplicity of new strains arising from a mixture of two parental types. The present work extends what was done previously and demonstrates that the process, which for the time being is called somatic hybridization, can result in new strains arising under natural conditions in the field.

*Mixtures of Red 111 and Orange NR-2.*

Further mixtures have been made from single spore cultures of the strains used previously by the senior author (Watson, 1957). The progeny from fifteen mixtures in all have been studied and the techniques have been similar to those adopted in earlier work. Strains unlike the parents were recovered from fourteen of the fifteen mixtures.

In Table 1 is presented a summary of the reaction types of the various strains that have been isolated from the mixtures. They have been tested several times on critical varieties and no evidence of instability has been found. The diversity of types is very striking. All the varieties representative of Stakman's series reacted differentially except Marquis and Khapli. The type 2 on Little Club is an unusual reaction to *P. graminis* var. *tritici* but is common if *P. graminis* var. *secalis* is placed onto seedlings of this variety. The virulence of this same strain on C.I.12632 is also unusual and shows clearly that genes for virulence have segregated in the progeny to give strains with a wider host range than that of the parents.

Two varieties with outstanding sources of resistance to the parents and their progeny were Marquillo and Khapstein. The virulence on Thatcher shown by strain NR-7 is difficult to explain at present, especially when a related variety Marquillo showed such a high resistance. Possibly all the effective genes of Marquillo are not present in Thatcher.

TABLE 1.

*Reaction Types shown by Fifteen Wheat Varieties when Inoculated with Red 111 and Orange NR-2 and Eleven Strains Resulting from Crossing them on Wheat Seedlings.*

Variety.	Parents.		Progeny.										
	Red 111.	Orange NR-2.	1	2	3	4	5	6	7	8	9	10	11
Little Club ..	4	4	4	4	4	4	4	4	4	2	4	4	4
Marquis ..	0;	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Reliance ..	0;	3+	0	3+	0	3+	0	3+	0	3+	3+	0	3+
Kota ..	0;	3	3	3	3	3	3	3	;	3	3	3	3
Spelmar ..	0;	0;	0;	0;	3+	3+	3+	;	;	3+	3+	3+	3+
Kubanka ..	0;	x	3+	3+	3+	3+	3+	3	2	2	x=	2+	3+
Acme ..	0;	0;	0;	0;	3+	3+	3+	;	3	3-c	x=	3	3+
Einkorn ..	0;	3+	0;	0;	0;	0;	0;	3	0;	0;	0;	0;	0;
Vernal ..	0;	3+	3+	0;	x+	0;	x+	3	0;	0;	0;	0;	0;
Yalta ..	0;	3+	;1	;1	;1	;1	3+	3+	;1	;1	3+	;1	3+
Bokveld ..	0;	0;	;1	;1	;1	;1	;1	;1	;1	;1	3 <sup>c</sup>	;1	3 <sup>c</sup>
Thatcher ..	0;	0;	0;	0;	0;	0;	0;	x <sup>+</sup> <sub>+</sub>	;	x+	;1	;	x+
Marquillo ..	0;	0;	0;	0;	0;	0;	0;	;	;1	;1	;	x+	x-
C.I. 12632 ..	0;	;1	;1	;1	;1	;1	;1	x <sup>+</sup> <sub>+</sub>	1+	3	;1	x	x-
Khapstein ..	0;	;1+	;1+	;1+	;1+	;1+	;1+	;1	;1+	;1+	;1+	;1+	;1
Strain designation ..	Race 111	NR-2	NR-4	Race 196	NR-3	Race 34	NR-5	NR-7	Race 54	NR-6	Race 222	NR-8	NR-9

In Table 2 are given the frequencies with which certain of the strains were recovered from various mixtures. Figures were not obtained from mixtures 1 and 2 and no red pustules were found on Marquis in mixture number 6. Two strains, race 34 and the NR-6, have tended to predominate in the progeny of the mixtures as a whole and the frequency of recovery of any one strain is most probably related to

TABLE 2.

*Frequency of Isolation of Eleven Strains as Progeny of the Cross Red 111 × Orange NR-2 when Different Screening Varieties are Used.*

Mixture Number.	Screening Varieties.	Strains Recovered and Frequency.
1*	Standard differentials.	Race 34, NR-3, NR-4.
2*	" "	NR-3, Race 196.
3	" "	Races 34 (3), 196 (6).
4	" "	NR-5 (1).
5	Emmer.	NR-6 (1), NR-7 (3).
6	Marquis.	Nil.
7	" and Emmer.	Race 34 (1), NR-6 (3), Race 222 (2), Race 54 (2), NR-8 (2).
8	" " "	Race 34 (1), NR-6 (13), Race 222 (2), Race 54 (2).
9	" " Kota.	Race 34 (1), NR-6 (9), Race 222 (1), Race 54 (2).
10	" " "	Race 34 (1).
11	" " "	Race 34 (7), NR-6 (2).
12	" " "	Race 34 (3), NR-6 (1), NR-7 (2), Race 54 (4), NR-9 (1), NR-8 (3).
13	Reliance, Marquis and Kota.	NR-6 (2).
14	" " " "	Race 34 (2), NR-6 (4).
15	" " " "	Race 34 (2), NR-6 (6).

\* The frequency of recovery of the strains from these mixtures was not recorded.

the variety used in screening. The table shows the varieties that were used in this latter process and, since these were so few in number, it is unlikely that a random sample of progeny was recovered from any one mixture. In mixture number 5, for example, where the isolations were made from Vernal Emmer, three of the four red pustules found were virulent on Emmer. To obtain data on the relative frequency of occurrence of various strains in the progeny the technique has been varied in experiments now being undertaken.

The data from Table 1 clearly confirm and expand the previous results (Watson, 1957) in which two cultures avirulent on Arnautka, Mindum, Spelmar and Acme gave rise to progeny to which these same varieties were susceptible. Since ability to attack these varieties has been found to behave as a dominant factor (Johnson, 1954) this would not be expected. The possibility of genes for pathogenicity other than those reported by Johnson, being associated with the reactions on these varieties, must not be overlooked. Their presence in this material is suggested from the complexity of strains resulting from the selfing of red 111 on barberry.

#### *Progeny of Red 111.*

Johnson (1954) usually found that when strains avirulent on Arnautka, Mindum and Spelmar were taken through barberries the progeny were also unable to attack these varieties. While there were certain exceptions, it appeared that avirulence was controlled by a recessive gene. Teleutospores of red 111 were produced at St. Paul,

TABLE 3.

*Reaction of Six Varieties of Wheat to Red Race 111 and Seven Selected Strains appearing among the Progeny obtained from Selfing.*

			Red 111 Parent.	Selected Progeny Strains.						
Reliance	..	..	0;	4	3	4	0	4	4	4
Arnautka	..	..	0;	3	3	x	0	3	0	4
Mindum	..	..	0;	3	1	1	0	x	0	1
Spelmar	..	..	0;	1	1	3	0	3	3	0
Acme	..	..	0;	;	3	1	3	1	3	0
Einkorn	..	..	0;	;	;	1	3	;	3	0

\* These strains were separated and the reactions taken by Messrs. Wilcoxson and Paharia and their help is gratefully acknowledged.

Minnesota, U.S.A., and used by the senior author to infect barberries. Among the resulting progeny were several strains virulent on one or other of the three above varieties and Acme (Table 3). From this table it is apparent that the parental strain red 111 is carrying genes for virulence on these varieties of *Triticum durum*. The effects of such genes only become manifest with segregation and recombination in selfed progeny.

It is suggested that some process, still unexplained, has resulted in the segregation of these same genes following the somatic hybridization that has taken place in the experiments reported herein. The segregation of these genes has resulted in the isolation of strains to which the varieties of *T. durum* are susceptible. The occurrence of unexpected strains among the progeny of red 111 could be due to genes for virulence associated with the varieties of *T. durum* being unlike those previously reported by Johnson. Since red 111 has been recovered as progeny of the intervarietal cross *P. graminis* var. *tritici* × *P. graminis* var. *secalis* (Johnson, 1949) it is possible that the genes for avirulence of the latter on the *T. durum* varieties have become associated with the genes for virulence on these same varieties from *P. graminis* var. *tritici* in this strain. While these latter genes normally behave as dominants, it may be that they are hypostatic to the genes from *P. graminis* var. *secalis* and strains having both are avirulent on varieties such as Mindum. When segregation occurs the presence of genes for virulence on the *T. durum* varieties again becomes evident.



The data of Table 3 show further that virulence on Arnautka, Mindum, Spelmar and Acme is not controlled by the same gene or group of genes, even though the reactions of the first three are usually the same to any one strain. The data also show that the gene for avirulence on Einkorn and Reliance are each in a heterozygous condition. Such heterozygosity would explain how segregation in the somatic hybrids between red 111 and NR-2 could result in strains virulent and avirulent on both these varieties. It is similarly postulated from the mixing studies that the genes for virulence on Vernal Emmer are also in a heterozygous condition. However, on selfing red 111 no cultures virulent on this variety were recovered. Since such virulence is controlled by two genes (Johnson and Newton, 1940), failure to find Vernal Emmer susceptible may have been due to the small number of isolations made. In general the results obtained from selfing red 111 complement and confirm those obtained from crossing red 111 and NR-2 on wheat seedlings. They reveal the genetic constitution of strain red 111 which would theoretically make possible certain strains recovered as somatic hybrids when this race is crossed with NR-2.

*The Importance of Somatic Hybrids in the Field.*

The cultures red 111 and orange NR-2 have been selected for studies of somatic hybridization since they are well marked genetically and since their progeny can be so readily isolated. Both are essentially laboratory cultures of little practical importance. The results with them have illustrated important principles but they have not demonstrated the significance of the process under natural conditions in the field.

Strains of rust prevalent in the field usually do not have the appropriate characteristics for studies of this kind, and this fact probably accounts for the early failure of attempts to demonstrate hybridization between dicaryons. The heterozygosity of genes for virulence, the colour of the spores and the varieties suitable for screening are all important factors which, if possible, should be considered in the selection of material for this type of experiment.

A search has been made to find strains from New South Wales which would be suitable for this purpose and experiments have been carried out using two of them. One was an orange biotype of strain 21 Anz-1 and the other a normal red biotype of strain 222 Anz-2. Red 21 Anz-1 is now the most prevalent strain in the south-eastern part of Australia (Watson, 1957*a*). Strain 222 Anz-2 occurs in the northern part of the eastern wheat belt and has been prevalent in northern New South Wales and southern Queensland where varieties having stem rust resistance similar to that of Gabo have been grown.

Inoculum from single spore cultures of orange 21 Anz-1\* and of red 222 Anz-2 was increased separately on Little Club. It was then mixed mechanically and used to inoculate the variety Acme on which orange 21 Anz-1 is virulent and red 222 Anz-2 is avirulent. Red pustules occurring on Acme were then isolated and examined. The whole experiment was carried out in strict isolation and there was no evidence of contamination from outside.

In Table 4 are given the reactions on five wheat varieties of six strains that were isolated and it is evident that certain of them combine the characters of both parents although none of the progeny was orange. Strains A, C and E were virulent on C.I.12632, an unusual feature to be found in local strains, and strain F combined in the one strain virulence on Celebration and on Yalta. B and D were unlike either parent and the whole progeny of six new strains suggests that somatic hybridization has occurred between orange 21-1 and red 222-2. On Stakman's differential series, strain F conforms most closely to race 21, and since it resembles strain 21 Anz-1 except that it has the additional ability to attack Yalta, it has been designated 21 Anz-2. Its appearance among the segregates was of considerable significance.

As reported earlier, red strain 21 Anz-1 was first recorded in 1954. It spread rapidly in the south, and although it was common in the north of New South Wales

\* Our colleague Dr. E. P. Baker first isolated this strain in 1955 from Griffith in New South Wales.

the rate of increase was retarded by the extensive cultivation of Gabo and varieties with a similar type of resistance to it. Crops of the susceptible variety Bencubbin were in sufficient quantity in the area to enable it to build up, and this variety from time to time during 1955 and 1956 was found simultaneously infected by 21 Anz-1 and one or other of the prevalent strains in the area 126 Anz-3 and 222 Anz-2. Facilities were available on a field scale to enable hybridization between strains 21 Anz-1 and 222 Anz-2 to occur.

Late in 1956, strain 21 Anz-2 was first recorded from Woodburn in northern New South Wales from material sent in by Mr. H. Farrelly on Federation wheat. Since it attacks Yalta, the varieties Gabo, Koda and Charter which have a similar resistance and which are widely cultivated in the area are susceptible. This fact as well as the remarkable survival and competitive ability of strain 21 Anz-2 have enabled it to become by far the most prevalent strain of *P. graminis* var. *tritici* in northern New South Wales and southern Queensland.

TABLE 4.

*Reactions of Five Varieties of Wheat to Two Parental Strains of Rust and Six of their Progeny Resulting from Mixing them on Wheat Seedlings.*

	Parents.		Progeny.					
	Orange 21-1	Red 222-2	A.	B.	C.	D.	E.	F.
Little Club ..	4	4	4	4	4	4	4	4
Reliance ..	0	3+	3+	0	0	3+	3+	0
Acme ..	3+	x=	x+	x+	x-	3 <sup>c</sup>	x	3 <sup>c</sup>
Yalta ..	;	3+	3+	;	;	3+	;	3+
C.I. 12632 ..	1+	1+	x+	x-	3 <sup>c</sup>	1+	3 <sup>c</sup>	1+
Celebration ..	3+	;1	x+	3+		x=		3+

While the authors hold the view (Watson and Luig, 1958) that 21 Anz-1 originated on barberries in Tasmania, the isolation of 21 Anz-2 in the north suggests a separate origin for this strain. The production of strain 21 Anz-2 in the laboratory by hybridizing two strains of rust almost identical with those occurring in the field shows that somatic hybridization in the field between red 21 Anz-1 and red 222 Anz-2 must be considered as a probable mode of origin of this strain.

#### *Discussion and Conclusions.*

Somatic hybridization must now be assumed to be an important mode of origin of new strains of wheat stem rust. Certain parental strains can be shown to undergo the process readily so that there is no difficulty in isolating the progeny from repeated mixtures. Other strains which are not so well suited for the experiments do not allow progeny to be isolated with such frequency. At this stage, however, the frequency of hybridization between different strains cannot be given. It is possible that some strains will hybridize frequently, others less readily and some seldom or not at all, as Holloway (1954) has found for strains of *Neurospora crassa* with regard to asexual reproduction.

The results from this experiment show that nuclear exchange cannot satisfactorily explain the origin of new strains among the progeny of red 111 and orange NR-2. The diversity of types exceeds that possible by this process alone. The authors conclude from the difference in frequency of the strains isolated in the progeny and their wide range in pathogenicity that a process of segregation and recombination is involved. So far no evidence is available to show that nuclear fusion has occurred between the parents but the hypothesis suggested is that such fusion does take place. The fusion follows anastomosing between the hyphae of the different parental strains (Nelson *et al.*, 1955) and the nuclei of opposite sex take part. The fusion nucleus is short-lived and segregates immediately giving rise to other nuclei with different

gene combinations. By an association of these nuclei, dicaryons are formed having new combinations of genes for virulence which become evident as new pathogenic strains. The exact sequence of events cannot be given as Pontecorvo (1956) has done for the Ascomycetes but so far we have been unable to isolate an unstable strain the equivalent of the diploid in *Aspergillus*.

While the exact mechanism making somatic hybridization possible must await further work, the synthesis of new strains important in the field by mixing inoculum of prevalent natural strains has great significance. Sexual hybridization and mutation must now be supplemented by somatic hybridization to make three important causes of variability in *P. graminis* var. *tritici* under natural conditions. A particular strain of rust, however, need not necessarily arise in one way only. When only a single step in pathogenicity is involved mutation can account for changes in virulence (Watson, 1957b). Strain 21 Anz-2 could have arisen from 21 Anz-1 by mutation for pathogenicity on one variety but we have not attempted to produce it in this way. In 1958, however, a strain conforming with 21 Anz-2 has been isolated from barberries in Tasmania. As 21 Anz-1 is the prevalent strain on the island and as a 21 Anz-2 had not previously been isolated there it appears likely that 21 Anz-2 is a segregate obtained from selfing 21 Anz-1 on the alternate host. Strains conforming in their reaction type with a well-known strain such as 21 Anz-2 but having evolved by different means will ultimately be shown to have some but not all of their genes in common. Hence minor differences in reaction type will always be found among them.

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